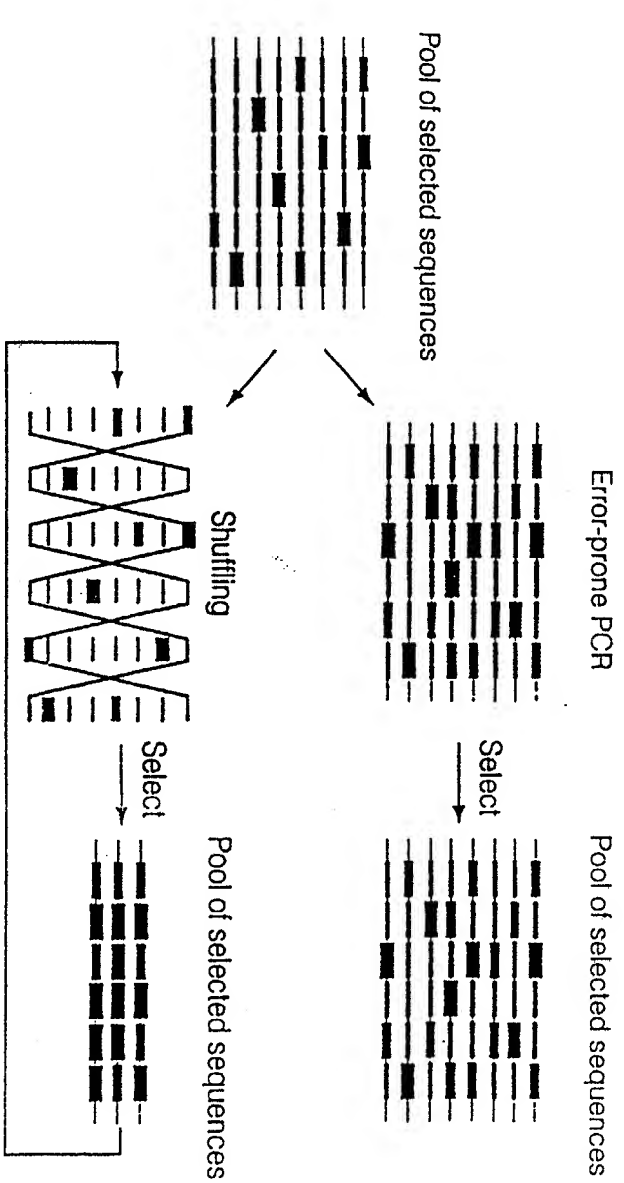

FIGURE 1

Sexual PCR

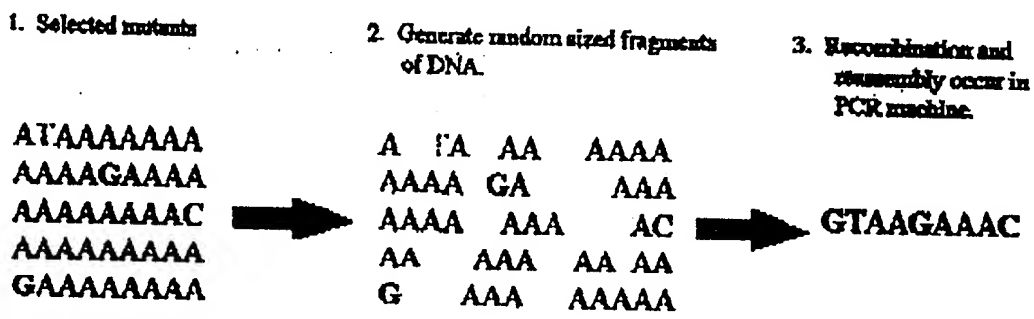

FIGURE 2

DNA ADDUCTS FOR SEXUAL PCR

1. RANDOM PRINTERS ARE USED TO SIMPLIFY TEMPLATES PRETREATED WITH DNA ADDUCTS.
2. ADDUCTS CAUSE PREMATURE TERMINATION OF EXTENSION BY BLOCKING THE POLYMERASE.
3. RANDOM SIZE FRAGMENTS ARE CREATED BY RANDOM PRINTING AND PREMATURE TERMINATION, NOT BY DIGESTION.
4. DNA FRAGMENTS ARE READY FOR SEXUAL PCR.

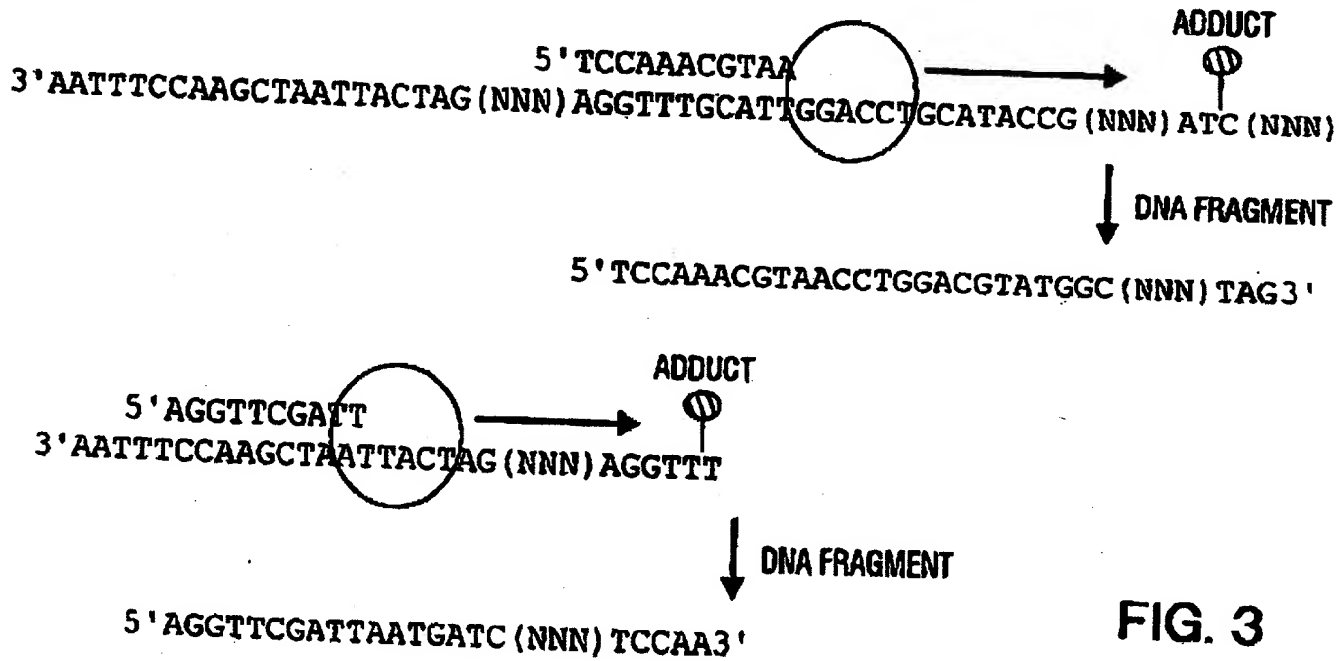

FIG. 3

DNA Adducts

Aristolochic acid 1
Aristolochic acid 2
2-Amino-3-methylimidazo(4,5-f) quinoline
2-Amino-1-methyl-6-phenylimidazo(4,5-b)pyridine
2-bromoacrolein (2BA)
7-bromomethylbenz(a)anthracene
benzo(a)pyrene
benzo(a)pyrene diolepoxide
Mitomycin C
camptothecin
(+)-CC-1065 (from Streptomyces zelensis)
N-hydroxy-4'-fluoro-4acetylaminobiphenyl
trivalent chromium
aromatic amines
platinum(II)
UV

FIGURE 4

CREATING DNA ADDUCTS USING U.V. LIGHT

1. IRRADIATE POOL OF TEMPLATE DNA WITH U.V. LIGHT.

5'AGATTAAGGAGTCCGTAAGGATT3'
5'AGATTAAGGAGTCCGTAAGGATT3'
5'AGATTAAGGAGTCCGTAAGGATT3'

2. CROSS LINKS IN THE DNA WILL BE INTRODUCED BY THE U.V. THESE CROSS LINKS WILL STOP TAQ POLYMERASE EXTENSION.

5'AGATTAAGGAGTCCGTAAGGATT3'

5'AGATTAAGGAGTCCGTAAGGATT3'

5'AGATTAAGGAGTCCGTAAGGATT3'

3. USE RANDOM PRIMERS ON CROSS LINKED DNA AND EXTEND WITH TAQ POLYMERASE

← 3'AGGCAT5'
5'AGATTAAGGAGTCCGTAAGGATT3'

← 3'CCTAA5'
5'AGATTAAGGAGTCCGTAAGGATT3'

← 3'CTCAG5'
5'AGATTAAGGAGTCCGTAAGGATT3'

4. TAQ EXTENSIONS ARE BLOCKED BY U.V. ADDUCTS. THIS CREATES RANDOM SIZE FRAGMENTS READY FOR GENE SHUFFLING

3'TCTAATTCCTCAGGCAT5'
3'AGGCATTCCTAA5'
3'AATTCCTCAG5'

FIG. 5

Reassembly of DNA fragments

Edd Lee

Fig
lane 1- Isolated DNA fragments of mutant OCSa alkaline phosphatase gene, length of ORF is 1.8kb
lane 2- 1kb ladder

FIGURE 6A

Fig
lane1-First round of reassembly, 1kb products have formed.
lane 2-Second round of reassembly
Predominant band at 1.8kb is the full alk phos ORF.
Reassembled product is ready for amplification, cloning and screening.
lane 3- 1kb ladder

FIGURE 6B

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Figure 7

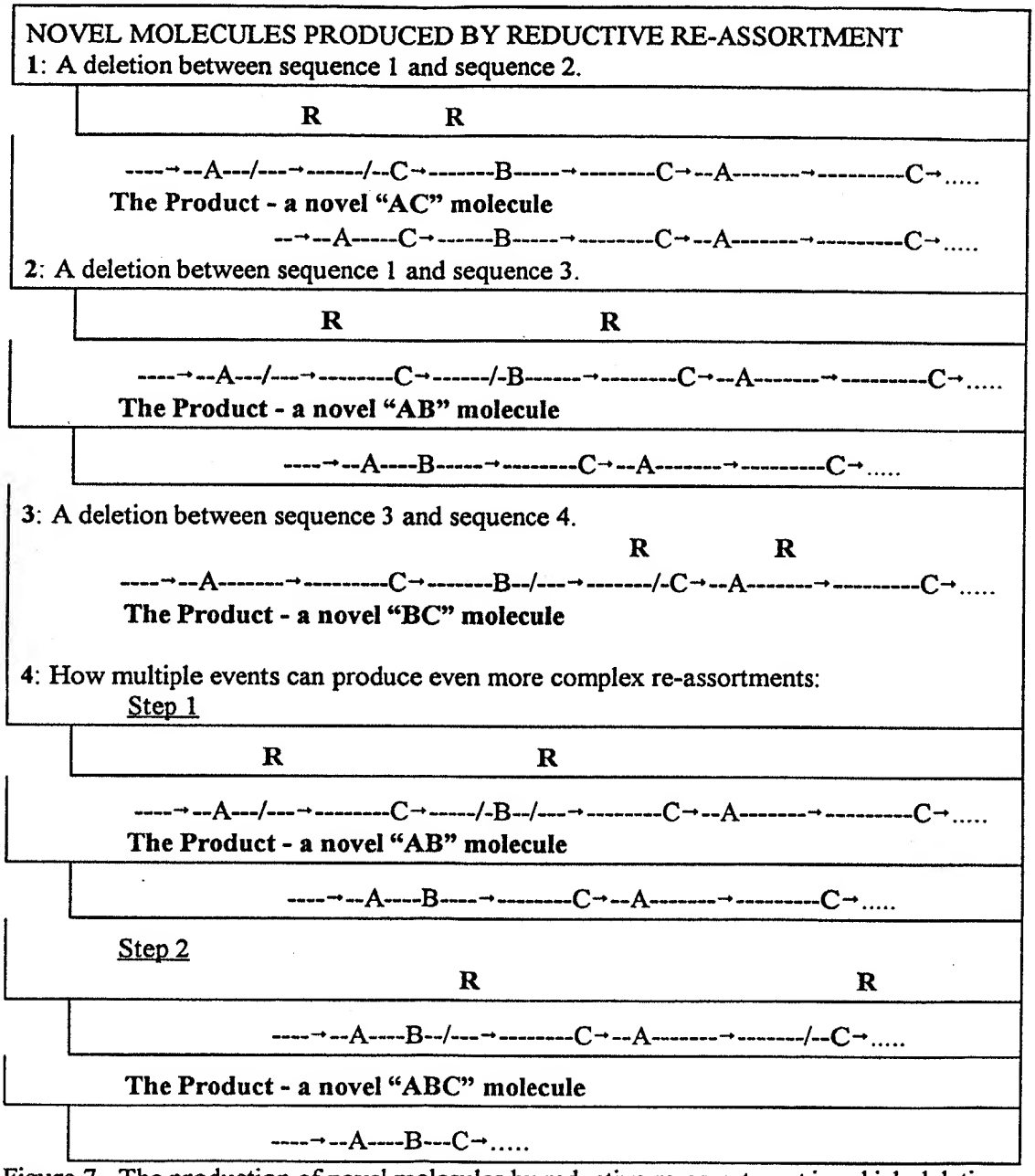

Figure 7. The production of novel molecules by reductive re-assortment in which deletions mediated by consecutive sequences result in the production of novel molecules. The inherent instability of repeated sequences drives this process. Multiple changes can occur within a single repeat unit through the reiterative nature of the drive to reduce the repeated index (RI).